February 2011 Issue | David Sinclair, PhD Department of Pathology Harvard Medical School

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Welcome to *Functional Medicine Update* for February 2011. The Methuselah dilemma. Ponce De Leon. Life forever. Pro-longevity. Anti-aging. These are all interesting terms, aren't they? They conjure up all sorts of things youthful. This concept of longevity and no senescence has been part of literature, art, music, and poetry for hundreds of years (probably thousands of years). It has also been part of the human dilemma: Why do we get older, and as we get older why do we age, and why do we ultimately die?

Even with all that work, and all that thought, and all that wonderful writing, literature, and creative contribution, the answer is still, "We don't know." But there are very remarkable steps being made at the cellular/biological, molecular/genetic, and biochemical levels that are starting to unravel this very complex story. The answers may not all be in, but what we are starting to witness is that many age-related decrements are associated with the loss of function: loss of function, as the patency of our book, of life has been water-spotted, defaced, or damaged over the course of living so that we're no longer able to translate messages effectively from our genesto the function of our cells.

The lack of appropriate translation ultimately can give rise to dysfunctions that are seen in distorted metabolism, and pathology, and things that we associate with apoptotic cell death and senescence of cells. Loss of function biomarkers as well as biochemical markers can be measured in the individual: loss of strength, flexibility, endurance, hearing threshold, vibratory sensation, cognitive function, number recall. All of those kinds of things are whole-organism manifestations of the cellular loss of function.

Is Progress Being Made in the Field of Aging Research?

What do we do about this? Where is the field going? Is there any hope? Are we making progress? These are remarkable questions that tie back to the substance of function. There has never been a monozygotic gene that has been identified to be associated with death, *per se*, nor is aging necessarily a prescription to disease; there is clearly a very strong association between aging and disease, but we can't say that aging in and of itself is a disease process. In our own experience, we may know people whose birthdays may number 90, and whose function may be that of an average 60 year old. And then we know the converse:

people whose birthdays are 50, who are performing biologically like average 90 year olds. There is a correlation between age, and disease, and health, but it's not a direct connection.

What is the variable that gives rise to our outcome as an individual--our functional capacity--as our birthdays come and go? It's that question that we are going to be discussing with a person who, for me, represents the new era of cellular biology, molecular genetics, and molecular medicine. His name has almost become—at a young age—a buzzword.

We're going to spend the better part of 45 minutes of this issue talking with this leader, this vision tender, this paradigm shifter, about his contributions to science and his thoughts on gerontology, geriatrics, and loss of function with age and how it relates to disease. From that, I will try to weave some threads of takeaway value that might be guides for our future. That's a big promise to fulfill, but I think it will be done very adequately by this month's clinician of the month. Let's turn to our interview.

INTERVIEW TRANSCRIPT

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Here we are in 2011—another chapter in the history of Functional Medicine Update. For those of you who have been longstanding listeners and participants, you know this is my favorite point in every issue issue. This month we are absolutely, I think, going to be stimulated with the story of our expert, Dr. David Sinclair. For many of you who have kept up with this field, the name is probably very familiar. Dr. Sinclair is an Associate Professor in the Department of Pathology at Harvard Medical School. I want to compliment him because he is recently tenured, which is no easy task at Harvard Med—and tenured at a very young age, which is another major accomplishment.

David received his Bachelor of Science in Australia. He is originally from Australia. He received a PhD in biochemistry and molecular genetics from the University of New South Wales, a wonderful school. I have been on that campus and done a number of lectures over the years; it is a great institution. He moved from there to do his post-doctoral work at MIT, and then later he joined Harvard Med to work in this whole area of molecular gerontology in the Department of Pathology.

Dr. Sinclair is known for being the father of the resveratrol revolution. But there is so much more to this story than resveratrol; there is so much more that Dr. Sinclair has brought to this field that has opened up

the domain of healthy and successful aging, age-related diseases and modification thereof, and this whole biological senescence process that we are going to be discussing with him.

Dr. Sinclair—David—thanks so much for being available for us today on Functional Medicine Update.

DS: You're welcome, Jeff. Thanks for having me on.

JB: Let me start down the path here. I've made some mental notes of the people I've had the privilege of speaking to over the last 29 years of doing this, and one of the personalities that came to light immediately that bears a little bit on your work is Roy Walford, a medical doctor who started in 1954 at UCLA in the Department of Pathology at the medical school. Many of us know him as the biospherian in Biosphere 2 from 1991 to 1993. He was very actively involved in kind of putting proof to Clive McCay's work at Cornell on calorie restriction. I know your work has borne on trying to discover some of the molecular mechanisms that relate to this. Can you give us some historic context as to how you took this early stuff from Clive McCay and Roy Walford and others and wove it into this extraordinary tapestry that you've developed in molecular gerontology?

Dr. Lenny Guarente and Yeast Research: Important Discoveries

DS: Sure, I'll give it a shot. The names you mentioned, among many others, laid the groundwork for the research we still do in my lab. Of course I'm standing on their shoulders, and Roy contributed a great deal to our early understanding of how important this dietary calorie restriction is to understanding not just the aging process itself, but how we might actually slow it down. It's still regarded as the most robust way to slow down aging in mammals.

The way I got involved in this actually goes back to when I was a teenager. I went into college, and I was dismayed at the thought that our generation may be the last one to live a normal human lifespan, and in the future this new wave of what we were calling genetic engineering would give rise to knowledge about aging and being able to slow it down. I was a little bit younger and more selfish in those days, but it really disturbed me that perhaps I was born just one generation too early to see this happen. So I was driven by the thought that maybe I could not just contribute to my children's and their children's health, but also, perhaps if I was really fast, have an impact on people like myself who are currently alive and were born in the 20th century.

I finished my PhD and looked for someone who was doing the cutting edge research in the biology of aging, and I was very lucky to have met—in Australia—a fellow who is now still one of my closest friends, Lenny Guarente, who at the time was just starting up work to understand why yeast cells grow old and what we might do about it—what we might learn from these simple yeast cells. To me, that seemed like the best way to go about studying aging, because after decades of work on humans and even mice, I thought very little progress had been made at the molecular level, and it's not because these people like Roy Walford weren't smart, it's just that the system is extremely complicated. In yeast, we thought we could grab hold of it. And that's what we did from 1995 to 1999/2000. Lenny's lab (I was part of the team) made some extraordinary discoveries; at the time even we didn't realize how important they were. We were having a lot of fun just figuring out why yeast grow old. Lenny and I published a paper in the journal Cell that identified a cause of aging in yeast cells (it turns out it's got to do with their genomic instability).[1]

The Discovery of Sirtuin Genes

The next big discovery came from another student in the lab who tried to slow down this genetic instability in yeast, and showed that a gene called Sir2 was able to do that, and he extended the life of those yeast cells. So if you fast-forward to the present day, these sirtuin genes—as they are called—that we co-discovered back about 11 or 12 years ago, are now known to be found in all life forms, and that includes bacteria and plants. What we think is going on, and there is a lot of evidence now, is that they are major players in getting the health benefits of this diet calorie restriction. What we have been able to do is to not just genetically manipulate mice, but even feed them moleculesl (including resveratrol) and give the physiology of calorie restriction without the mice having to actually diet. We've published this, and we've made inroads into clinical trials trying to give the benefits of dieting and even exercise to mice and to humans with a pill.

That's where we're at, and that's where I came from.

JB: That's a really beautiful, succinct summary of a lot of hard work, and a lot of insight, and a lot of midnight oil. Genomic instability reminds me of another person who is actually one of your fellow Australians that we've had a chance to talk with a couple of times, and that's Michael Fenech at CSIRO down in Adelaide. Mike was talking about histone compatibility, histone integrity, the nucleosome and how it is constructed, and what happens when you have imperfections in the protective coat of our book of life material (our genome). The sirtuin discovery that you and Lenny Guarente made really relates also, it seems, to this histone integrity, in part due to the fact that the sirtuins are NAD-dependent histone deacetylases. Can you tell us a little bit about that whole connection between sirtuins and their function, and genetic stability/instability?

Sirtuin Genes and Genetic Instability

DS: Sure. It is a fascinating area. The discovery in yeast told us that these enzymes control the chromatin, which is the building block of the chromosomes—essentially the way DNA is wrapped up around protein. It does that specifically by clipping acetyl groups off the histone tails. It is a very elegant reaction, and requires NAD, which is a cofactor in metabolism. We now know that the levels of NAD and the amount of sirtuin activity in a body—we know in mice and it looks like it is true for us as well—varies during the day; it even varies with our circadian rhythms and can get out of whack when you are jet-lagged. So this is very central. But getting back to chromatin, what we have since discovered is that this is also true for mammals. We looked at mice and we had a paper in 2008 in Cell that showed that SIRT1 does two things: it controls the way genes are turned on and off (which is a major function of chromatin), but also we found that this protein (SIRT1, which is the mammalian version of sirtuin yeast) is involved and required for efficient DNA repair (when the chromosome is broken; when DNA is broken).[2]

So we've got two functions: its control of genes (whether they are turned on and off), and we've got it also repairing DNA. What we found was that if you damage a cell, with too much damage SIRT1 becomes distracted, going off to repair all the damage and the genes that it normally would control and maintain in a youthful pattern of expression ends up getting disrupted. And we were able to slow down the changes with aging by giving a mouse more of this enzyme. Essentially we call this the "RCM hypothesis." It stands for "Relocalization of Chromatin Modifiers." It is a way to explain why the way our genes are switched on and off when we are young ends up getting dysregulated as we get older and no one really understands why. We're saying it's actually because DNA damage accelerates that process by distracting proteins that would otherwise control our genes.

So that's where we are. We are hopefully going to be able to reverse aspects of aging one day because that's the implication: if only we can send SIRT1 back to where it should be instead of being distracted, we could make organs function like they used to when we were young.

Connecting Aging Research to the Xenohormesis Concept

JB: That takes me back again to a very extraordinary part of your work. Over the last 10-plus years you have put together more than 110 publications in top-tier journals. You haven't been sitting around waiting for things to do, that's for sure. One of the many—I call them seminal—publications, from my reading, is a publication on xenohormesis.[3] It reminded me of an interview that we did some time ago with Ed Calabrese, who has been kind of a father and maybe a voice in the wilderness, to some extent, concerning this concept of hormesis (some small agent having unexpected large effects on a system). I think your development of this concept of xenohormesis is a seminal platform concept in biology. I think the article that you had in Cell was just one of those really "aha" articles that should be mandatory reading for every medical student and every bioscientist. Could you tell us a little bit about what xenohormesis is, and where this concept came from and your support for it? I think it is a fascinating concept.

DS: Sure, thanks. I should, at the outset, also give due credit to my co-author, Konrad Howitz. He and I have worked on this theory for a number of years, and he was actually the first person who had the initial insight: why would it be that a molecule like resveratrol can hit so many different proteins and targets in a cell, seemingly for the benefit of the organism? Another example of that would be aspirin. Why is aspirin so beneficial in, as people have found, many different targets? Aspirin seems to modulate pathways just in the right way that is healthy. What Konrad and I have been developing is the idea that this is no accident—that it really is our body's way of getting a sense of the environment and hunkering down in advance of food shortage, for example.

The concept, at its core, is that early life forms evolved to sense other life forms when they were stressed so you could get a heads-up about deterioration in your environment. What we proposed was that when plants are stressed (as an example—it probably works between very different organisms, not just plants, but fungi and others), they make high levels of secondary metabolites (resveratrol, aspirin, many others). And what we think is going on is that we have evolved to sense those chemicals because we are consuming plants, we're consuming other organisms over time, and we can get a sense of how our food supply is actually doing in the environment.

You could imagine a scenario where one organism can sense whether its food supply is stressed and another organism cannot, and the one that can sense it through what we call xenohormesis is able to prepare in advance for the loss of its food supply, or some other stress that is coming that it cannot sense itself but other species can sense, and that it gets ready for the stress, and the other organism is oblivious. The oblivious organism ends up dying out, and you are left with organisms on the planet that can sense other species when they are stressed.

JB: I think there is a beautiful poetic social metaphor there. I know you have spoken to it or related to it in some of your writings—that this almost speaks to the concept that has been often, in genetics and evolution classes, considered a non sequitur, and that is co-evolution, where we are actually getting cooperation among organisms by one organism doing a lot of work for another and getting a benefit in return. It's a very interesting concept. I guess in some senses, we could even look at tryptophan and its conversion to

niacin in the body as being somewhat like that as well, because niacin is a conditionally essential nutrient—our body makes it to some extent, but it's nice when plants do more of it for us. It seems like a very interesting concept that talks about network biology.

DS: Yes, a lot of people have come up to Konrad and me and said that this has explained a lot of things that have been just brushed aside as a coincidence. It's a very hard thing to prove. We did try in the lab—a little bit—to prove this and never ended up publishing something. We were trying to feed aphids arabidopsis plants that had been stressed out by light. Although we made some progress and had some early data, we never finished it (or I should say that the student gave up because it was an extremely difficult project). Right now, what we rely on is a whole body of associations that support this idea, but we don't have the proof yet, and I'm hoping that either my lab one day or someone else will come up with that clear evidence.

Contrasting between the Pharmaceutical and Xenohormetic Models

JB: Let me, if I can, play off that for just a second. I know that this kind of xenohormetic model ("xeno" meaning foreign, and "hormetic" meaning small things having bigger effects than expected) is a slightly (maybe even significantly) different model than the pharmaceutical model from which many of our medicines are derived, which is a one disease, one biomarker, one molecular target model. It is kind of a linear model. It has led to the development of a very robust Physician's Desk Reference (PDR), with a lot of new-to-nature molecules that do specific things, but it raises a question as to whether there is a molecule that actually does just one thing, or whether we have pleiotropic effects that then give off target influences that give rise to all sorts of other things that we previously didn't understand because we don't know what we don't study. Can you comment a little bit on the pharmaceutical model as it contrasts to the xenohormetic model?

DS: Yes, sure. The xenohormetic model predicts that a single plant molecule could hit maybe a dozen or more different proteins and modulate them in just the right way to provide health. As I mentioned, we see that in botanicals that are used as medicines all the time, and even medicines that are natural, such as metformin for diabetes. The alternative view, which is predominant in the pharmaceutical industry, is that you should make a drug that hits just one target, because if it hits more than one, there is much greater chance for what are called off-target effects and toxicities. Which is true, but that's only because we end up—in the pharmaceutical industry—making synthetic molecules that we have not evolved, ever, to experience.

They are really two different worlds and I don't think it is correct for one to say negative things about the other even though they do, because they are two different worlds: one is synthetic and never experienced, and the other is natural, where organisms have been bathed in these types of molecules for a billion years. What it actually predicts, though, is that the pharmaceutical industry could identify medicines, particularly from plants that have been stressed. I'm unaware of anybody who is taking that strategy—that if you wanted to look in the natural world for a new medicines, you should isolate molecules particularly from those plants that have been stressed.

The other thing that is interesting is that the agricultural industry, except for perhaps the organic side of things, tries to make plants as happy as possible (they tend to grow faster). But as Ed Calabrese would say, if you give a little bit of stress to plants they can actually do better. What we believe with xenohormesis is that you would have a healthier food supply if you did stress plants just before picking

them. And actually if you look at the best red wines in terms of flavor and even health (levels of resveratrol as well), these wines come from grapes that are under stress, either from fungal attack or dehydration. But we don't typically apply those types of approaches to our other foods.

JB: You know, one of the colleagues of yours at NIH that has referenced your work extensively and is following this same theme is Mark Mattson—I'm sure you know him—in the neurohormesis area and his work on neurodegenerative diseases and phytochemical modulation of neurological function. Does his approach seem consistent with what you have observed from your work?

DS: Sure. They go hand-in-hand—the idea that a little bit of self-stress is good for you—is really not just the basis for his work and mine, but the whole field of calorie restriction now has realized that the reason that this diet works (in the brain, in the body, for metabolism) is because it is invoking a perceived stress on the body. And what's happening is it is not just changing metabolism and slowing it down, which was the early theory, but it is invoking a stress response in the animal. By that I mean it is turning on particular pathways that we are now characterizing, like the sirtuin genes that I mentioned earlier. That actually now means that it is feasible for Mark and myself to find particular genes that can mimic the benefits of these stresses without actually having to experience them. I think that's a real breakthrough in concept as well as practical approach.

Sirtuins and Prion-Related Diseases

JB: Of the many papers, two that really struck my fancy was a paper looking at prion-related diseases and the influence that sirtuins might have (or sirtuin activation and/or calorie restriction).[4][5] If I think of damaged chromatin, then I also can think of damaged protein. Prions are mis-folded proteins. Can you tell us a little bit about that? That seems like a fascinating part of the story as well.

DS: A number of labs now have realized that the sirtuin genes don't just control histones and the way genes are turned on and off, but they actually control other defense pathways, like antioxidant defenses and also, as you mentioned, protein mis-folding defenses. That's an area of extreme interest because what we know about prion diseases and also about the aging process itself is that mis-folded proteins are a key cause. We're also looking intensively at Alzheimer's disease and finding that the sirtuin genes—at least 2 out of the 7 we have—are highly neuroprotective, enabling the body to get rid of mis-folded and aggregated proteins, like A-beta.

So this is an area that is extremely important, and actually it goes back to the days of the yeast cells, where researchers found that the original gene, Sir2, doesn't just control genomic stability, which is what we worked on, but also that this Sir2 protein is able to detect damaged proteins (oxidized proteins), and prevent the offspring from getting those proteins, so that the offspring are rejuvenated. There is still a lot to figure out. We don't know how Sir2 does that in yeast, and we certainly don't understand much more about mammals as well. But I think the important point, Jeff, here is that these sirtuins do a lot of things, and that's what you would expect of a gene that underlies the benefits of caloric restriction and can actually slow down aging.

JB: I'm thinking back, also, to my kind of very naïve understanding of the etiology of Werner's syndrome (or Werner's disease), which is a precocious aging where children with this genetic issue end up often going through what appears to be the whole senescent process by their teenage years. As I recall, one gene that has been located that relates to this has to do with helicase, which has to do with the tertiary

structure folding of proteins. Is there any connection at all between this precocious aging and helicase and the sirtuins, or is that a stretch of the story?

Sirtuins and Telomerase Activity

DS: It's not a stretch at all. In fact, I read a paper—at least the abstract of a paper—a few days ago that showed that resveratrol, and ostensibly SIRT1 that it's targeting, is able to upregulate the activity of the Werner's protein, as well as telomerase.[6] I think many of your listeners will know telomerase is the enzyme that extends telomeres. And the reason that's important not just Werner's syndrome but also in normal aging is there is increasing evidence that telomeres are important for diseases. And actually some of the early work we did in my lab was to show that the Werner's syndrome helicase is necessary for the maintenance of telomeres in yeast cells, and it looks like that is also true for patients and the problem that leads to their symptoms is rapid telomere erosion and hence rapid aging. But there are a lot of other problems, of course. They have defects in general DNA repair. But also, it is known that the SIRT1 protein (the enzyme) can regulate the Werner's helicase directly. So there are very clear links between what I work on and premature aging diseases (progerias, like Werner's syndrome).

Sirtuins and Epigenetics

JB: That's fascinating. I want to go back to this chromatin story with SIRT1 for a second. We talked about SIRT1 being an NAD-dependent histone deacetylase and it has to do with the acetylation/deactylation of histone protein, which then raises an interesting question to me, because that would be—I guess we would call it—an epigenetic type of regional specificity. And when we get into epigenetics, then we translate the portion of our book of life—our human genome—that is readable or unreadable based upon whether it is methylated or acetylated. That takes us into things like Randy Jirtle's work with folate and B12 and what they found in the Agouti mouse, or what Moshe Szyf is looking at at McGill as it relates to kind of psychosocial impacts on the epigenome, or Mike Skinner at Washington State who has been looking to the role of low level biocides on the epigenome. Clearly there appears to be a convergence or an interface between what you have done with SIRT1 and phytonutrient modulation of SIRT1, and some of these other people that are working in epigenetics. Can you tell us a little about this domain? It sounds like it is really advancing quite rapidly.

DS: Yes, it is. The paper that I was talking about that impinges on our work clearly says that the SIRT1 enzyme controls how we age not just based on what genes we inherited, but how those genes are expressed during aging, and that this can be accelerated by DNA damage by distracting this protein. In general, the field of epigenetics—as you mentioned—is exploding, particularly in aging, where we have realized that there is a lot more than just the genetics involved in predicting how we age. Some of the most interesting work, I think, is done by researchers who are studying the effect of maternal environment, and that even before you are born you may have a gene expression pattern set up in your cells that then predisposes you to particular diseases late in life. That's been done very effectively in rodent studies where rodents that are stressed, or, for example, fasted extensively during development, that these offspring go on to develop diabetes and obesity more often.[7],[8] That area is really exciting. It's a little scary because of how much of an impact your mother's behavior can have on you potentially, but it also—as you mentioned, Jeff—is important because as we head off into the genomics era, we have to realize that there is more to know than just reading your genome at the DNA level.

Research on Resveratrol and High Fat Diets

JB: One of your many important papers was the paper in Nature that talked about the role of high fat diets

on inducing distorted metabolic signaling and the relationship that it has to insulin resistance and inflammatory effects and how resveratrol could have a positive impact in ameliorating that.[9] It sounds like one of the stresses that we are talking about other than calorie restriction would be-- I guess you would call it--the malnutrition of overconsumptive undernutrition: too much of too little, or just overwhelming. Is this stressor of the American diet one of the precipitators for altered sirtuin regulation based on what you've found to date?

DS: You've hit the nail on the head. When we first proposed that a high fat diet could be at one end of the same spectrum as caloric restriction there were a lot of doubters. Now it is actually taken for granted that that's true, but about six years ago when we first started working on this and proposing it most people thought of caloric restriction (calorie restriction) as something rather special. How we viewed it was at one end you've got the high fat diet and at the other end you've caloric restriction and in between you've got a standard diet. What we proposed was that you could feed mice resveratrol if they are on a high fat diet and push them towards calorie restriction and end up having a fat mouse that looks more like a lean mouse. And then if you took a lean mouse and fed that one resveratrol we could push them towards caloric restriction. When we proposed that, like with most crazy scientific theories most people said, "This is never going to work." The postdoc, Joe Baur, who started the project, was convinced that his career was going down the drain. But what happened was it was borne out. The mice on the high fat diet ended up having the physiology of a lean mouse, even though they were still obese; their organs seemed to be oblivious to the fact that the mice were fat. And then when we fed resveratrol to the lean mice, they had the health, and the gene expression pattern, and the physiology of more like a calorie-restricted mouse.[10] That now is just taken for granted. It's funny to think back only five years ago how strange that sounded to most people and how risky the project was.

But you are also right: the high fat diet is a stress. One area that I'm thinking more about (or actually trying to understand) is why a high fat diet that most of us consume in the Western world accelerates the aging process. This is not a common view. In fact, a high fat diet has been thought of rather with derision by the aging community, but more and more I think people are coming to the realization—I certainly am—that a high fat diet actually is important to understand the aging process, and that it's an accelerator of that process. And if you wanted to know why I think that happens, my best guess right now (and we are testing this in the lab) is that the high fat diet turns down our body's natural genetic defenses against aging, like the sirtuin genes and others, and that if we eat a big hamburger, what we are really doing is turning off these defense pathways, and in the long run that accelerates aging.

Comments on the Hypoxia-Exercise Connection

JB: That's fascinating. One of the other things that has been discussed—and probably controversially—as a stress or maybe even as an activator (so they've got a lot of difference of opinion), is this whole exercise connection and how that also relates to hypoxia. I think you have done studiesin which you published the role of hypoxia on some of these signaling pathways.[11] Some people might say, "Exercise is good as a positive stress." And other people might say, "Exercise produces hypoxia, which is overly stressing and now we induce oxidative injury and genomic instability and so forth." What's your thought on the hypoxia-exercise connection?

DS: That's been one of the hottest areas recently. Not only are the sirtuins involved apparently in the benefits of dieting, but also of exercise. Some of the evidence is that when you exercise a rodent or even a human, the amount of some of these sirtuin genes goes up dramatically, as does their activating molecule,

NAD, and the effects of exercise can be recapitulated. We've done some of these in my lab by turning up these pathways, so you can mimic the benefits of exercise just with a genetic manipulation and get the boost in mitochondrial activity, and even down regulation of antioxidants. Really the challenge is to figure out what the right dose of exercise is beneficial. You mentioned that maybe you could overdo it. I think that's true. I think there is probably a balance where you can do the right amount of exercise without creating too much damage, and then that is a lasting effect that keeps these defense pathways up for the next few days, maybe even a week, and you get the benefits of the exercise without counteracting that with the damage that you can cause.

JB: I remember years ago talking to Dr. Edward Schneider, who I know you are familiar with. I think now he is the director of the Andrews Center of Gerontology at USC, but at the time I talked to him he was at the National Institutes of Aging as the director; he took over for Bob Butler. He made the comment at that point (probably two decades ago) that what had been learned about aging was moderation—that if you really looked at things, we have this curvilinear kind of response curve that is more parabolic: too little of something is not good, too much of something is not good, and at the zone of optimal function you've got the right amount of whatever it would be. Does that hold true, from what you've seen, as it relates to these modulators of sirtuins—resveratrol or whatever we are talking about, that there is some kind of a parabolic curve of dose response or effective response?

Dose Response is Still Undetermined

DS: Yes and no. Let's take SIRT1, for example, the one that we know the best, the sirtuin gene. It gets up regulated about 5-fold when you fast an animal overnight. So the "yes" to the question is that it is true that if you don't give enough of this gene—if you only up regulate it 50{56bf393340a09bbcd8c5d79756c8cbc94d8742c1127c19152f4230341a67fc36} or so, or you give resveratrol at a relatively low dose—you don't activate these pathways enough to get any meaningful of significant health benefits. The "no" part of it is that we haven't yet found an upper limit where SIRT1 is bad for you. With resveratrol, of course, you can make an animal sick by giving too much, but I haven't seen compelling data saying that too much resveratrol can be extremely harmful. The one exception to that was a clinical trial that was halted in multiple myeloma patients, where there was suspicion that there might be kidney failure.[12] But in normal individuals, and I'm aware of at least 200 that have been given gram quantities of resveratrol, there wasn't anything that was obviously bad. I'm not sure about the upper limit, but there is definitely a low limit where you need some sort of either fasting or some other type of stress or genetic manipulation that will get the levels up to where you can have benefits.

That's where we are. What we have done is we've made mice with different levels of SIRT1, and we are asking "What is the optimal level?" The question might actually be the wrong one because you may need to cycle the levels throughout the day, which is what happens naturally. What we're doing is fairly crude, just blasting the animal throughout its life with high levels of this gene. We'll have to see whether that works or it doesn't.

JB: I'm sure you've been asked a million times—in fact, I had a discussion with Rick Weindruch at the University of Wisconsin, who is a calorie restriction researcher about this question—about the level that would produce a meaningful effect on enhanced sirtuin-1 function in a mammal. He's working with monkeys, but he's also interested in humans as well. I guess there is a big controversy about that. We've seen a lot of people move in to exploit this by putting 50 or 100 milligrams of resveratrol in something and then citing your work. Do you have a sense as to whether we know where we start getting a gain of

function response to resveratrol?

DS: Yes. I certainly avoid making any judgments about products. There are a number of companies that get fairly angry with me if I say anything. But as a scientist I can tell you that the mouse experiments that we've done predict that if you scale up allometrically, the amounts that we see an effect with would be roughly 250 milligrams per day in a human, but that doesn't take into account things like the fact that we're a human not a mouse and there might be physiological differences in bioavailability. But if you just did the crude scaling up, it's about 250 milligrams.

Addressing Controversy about Resveratrol Research

JB: Every new theory, every new advance, every new discovery has its naysayers. We've seen this happen almost reproducibly with any paradigm-shifting concept. There was the Amgen work and the Pfizer work that was published recently, suggesting that the influence of resveratrol or sirtuin was really an artifact of the methodology of the fluorophore that was used in the assay.[13][14] I know you have gone back and re-looked at that. Can you comment on that really quickly?,

DS: There has been a lot of work just in the past year since those papers came out, and all of it, without exception, has been supportive of our view and our published work, which is that the resveratrol and also the synthetic molecules that have been made since directly bind to the enzyme and this fluorophore artifact is not the case. There is a lot of evidence, of course, and I would have to spend another hour with you, but actually some of the best evidence is that you can just take that fluorophore out of the assay and the experiment still works. So that's the best argument. It was actually a pretty simple experiment. That fluorophore is a bulky hydrophobic chemical, and there are bulky hydrophobic amino acids so we just replaced that fluorophore with tryptophan and the thing still worked. It's got nothing to do with a fluorescent artifact per se.

There is a lot of evidence now. In fact, just in the last two weeks I could point to three—maybe four—papers where the effects of resveratrol have been negated by removing SIRT1. And so the effect of resveratrol clearly requires SIRT1 in many physiological effects.[15][17] In my lab we're working on muscle metabolism and resveratrol clearly requires SIRT1 to change muscle metabolism. So I think that this last year has been helpful and it will continue to be controversial, but I think the weight of the evidence is swinging back in our favor.[16]

Mammalian Target of Rapamycin (mTOR) is an Emerging Area of Research

JB: I want to close with one last question. It appears one of the things that your extraordinary work has done is to provide a framework for the landscape of research in the area of molecular gerontology, which has been kind of in search of a Holy Grail for many years. I remember years ago talking to Caleb Finch, who was one of the early researchers in this area (I think back into the 70s, actually). He was always talking about how there will be ultimately a discovery that will help guide the research so that we will round up all these different theories—the Denham-Harman free radical theory, and antioxidants, and all these various things like the hormonal theory and so forth. It appears as if your work and that of your colleagues has started to provide a framework for this kind of structured approach towards understanding aging at the genomic and cellular level.

With that in mind, one of the things that seems to be emerging out of this field from this type of work is this whole question of intercellular signal transduction through kinase signaling pathways and how that

ties through central switching areas like mTOR. I find mTOR to be interesting when you think of the whole discovery of rapamycin as a fungi metabolite and how this "antibiotic" has now been found to have a very important role to play in this energy economy switching gene expression pattern and its connection with adenosine monophosphate kinase and nutrient sensing. Can you tell us a little bit about what your thoughts are as it relates to the trajectory of this field and the whole mTOR area? Is it part of this landscape that you are interested in?

DS: It absolutely is. There are really four horsemen of the aging field now: sirtuins, which we've talked about and which I work on predominantly; there is mTOR and rapamycin—rapamycin turns down TOR signaling; there is AMP kinase—you mentioned that; and then there is insulin signaling. These four systems, when I go to a meeting on aging there are some very strong personalities and typically someone will stand up and say, "My pathway is the one that is most important. Mine's the one that underlies aging and calorie restriction." And someone else will say the same about their pathway. It's becoming clear, at least to myself and a few others, that it's a crazy thing to argue whose pathway is more important because this is a network of environment-sensing genes that talk to each other, and if you tweak one, without exception the other three will change. The sirtuins lie right within this network and they control mTOR, and insulin controls it, and AMP kinase is both upstream and downstream of SIRT1. So the challenge for the field, besides calming down and realizing we're all holding the same elephant, is that we would like to figure out what's the best way to tweak these pathways that is beneficial without causing side effects, and that's really the challenge that my lab is working on right now.

JB: Could you give us just kind of a quick view to the future? You're probably the best prognosticator of what might be over the horizon that I know. Can you give us a sense as to what you think might be the light of the dawn here?

DS: Well, I could flip a coin. The reason I say that is there are clinical trials in progress right now. They are being run by GlaxoSmithKline, which (full disclosure) I consult for. I'm aware of how well these trials are progressing, and so far I remain just as optimistic, if not more than ever, about their progress through the clinic and into the market, but there is still a phase two, and of course anything can happen and probably bad things will happen; that's the nature of drug discovery. The best case scenario is that those drugs end up reaching the market for a disease. It may be an eyedrop, it may be…who knows, a pill, a suppository? I don't know what it will turn out to be, but that could be the first—and I think is most likely to be the first—application of this research (practical application).

But longer term, I think that we've turned a corner. I think that we now know how to manipulate health and aging. We know how to—at least in theory—control these major processes. Some drugs are already on the market, such as metformin. But longer term, I think that companies have realized that the study of these pathways and their integration and how they might control aging is important and that more people will work on them. The thing that a lot of people who don't think about this everyday forget or don't realize is that this is not about making a medicine to slow down aging. In fact, any company that tries to do that will probably go out of business. It's about making medicines that treat diseases of aging and even diseases in young people by turning on these defense pathways that we know at least in animals can extend lifespan and slow down aging. So the future, I think, is that best case scenario, within the next few years, the GlaxoSmithKline molecules will hit the market. I thing with drug discovery is we never know how that's going to turn out, but I think that the original goal of mine when I was in college may be achieved in my lifetime, which is that we may see the fruits of understanding how aging is controlled in

our bodies.

Recommended Reading about Dr. Sinclair's Research
JB: That's a very exciting and very optimistic perspective. For someone who would love to be able to read all of your 110+ publications but doesn't have the time, would you be comfortable with listeners getting a sense of your work via The Youth Pill, the David Stipp book?[18] I think it is a very fascinating kaleidoscopic overview of this whole concept that science is at the brink of this understanding aging revolution. Are you comfortable with that as being a good first start for many of our readers?

DS: I highly recommend David's book. I've known David for years and he has really done in-depth research and knows all the players and is a great writer. Yes, absolutely, that's probably the best way to start. For a little more detail, if you'd like it, I wrote a Scientific American article a few years back with Lenny Guarente, my mentor, that talks about longevity genes and how they work. So, that's in Scientific American.[19]

JB: I know you had a very nice paper—a response—in Science magazine in the summer of 2010 that re-looked at the sirtuin concept and gave us an update on what's happening.[20] We'll follow your publications very carefully because they are the pulse of really what is happening in this field. I can't thank you enough for spending this amount of time with us. This is more than fascinating. What we are hearing in a very measured way from you, and I know you are doing a very good job to meter your words, is really the birthing of a shifting paradigm that will really change the way of medical thought and medical therapy as it relates to this age-related disease process. David, thanks so much for being with us and thanks for your tireless work. It is extraordinary.

DS: Thanks. It's conversations like this that keep me going. Thanks a lot, Jeff, for having me on

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